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MORPHOLOGY, HEMATOLOGIC PARAMETERS, AND BEHAVIOR OF HEMOLYMPH CELLS OF THE QUAHAUG CLAM, *MERCENARIA MERCENARIA* [1]

DAVID A. FOLEY AND THOMAS C. CHENG

Institute for Pathobiology, Center for Health Sciences, Lehigh University, Bethlehem, Pennsylvania 18015

Molluscan hemolymph cells have been implicated in diverse functions, including an active role in internal defense against invading foreign materials, biotic and abiotic (see reviews by Feng, 1967; Cheng, 1967; Cheng and Rifkin, 1970). The immediate fate of soluble and particulate materials introduced into molluscs is well known. Specifically, injected particulate materials are phagocytosed (Stauber, 1950; Tripp, 1958a, 1958b, 1960, 1961; Feng, 1959, 1965a, 1965b, 1966a, 1966b; Arcadi, 1968; Cheng, Thakur and Rifkin, 1969; Pauley and Krassner, 1972) unless they are too large, in which case they are encapsulated (see reviews by Cheng, 1967; Cheng and Rifkin, 1970). It would thus appear imperative that the mechanisms involved in phagocytosis by molluscan cells be examined in detail if their role in internal defense is to be elucidated. Furthermore, it would be of interest to ascertain the ultimate fate of phagocytosed substances at the chemical level. However, before any detailed studies relative to these functions of molluscan leucocytes can be achieved, it is necessary that the numbers and types of hemolymph cells, as well as their normal behavior, be ascertained.

The purpose of this paper, therefore, is to describe the morphological and behavioral characteristics of the circulating cells of the hemolymph of *Mercenaria mercenaria*. Such descriptions are obviously essential as a preface to studies concerned with their function as related to internal defense. In addition, an attempt has been made to establish hematological parameters in this mollusc in order to determine their normal values and to ascertain whether these are interrelated. Samples of *M. mercenaria* collected from two different geographic areas were utilized to provide comparison.

Materials and Methods

Fresh specimens of the quahaug clam, *M. mercenaria*, from Buzzard's Bay, Massachusetts, and Great Bay, Long Island, New York, were obtained from a commercial source and held in the laboratory for 1 week prior to use. They were maintained at 19–20° C in a recirculating seawater system at a salinity of 20–25‰.

A 1.5 ml sample of hemolymph was withdrawn with a hypodermic needle and syringe from the anterior adductor muscle sinus of each clam by the method of Feng, Feng, Burke, and Khairallah (1971). One-half ml of the sample was immediately discharged onto a clean glass slide while the other 1 ml of hemolymph was emptied into a 10 × 75 mm test tube containing 0.03 ml of 50% glutaraldehyde.

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TABLE I

Total cell count and packed cell volume of Mercenaria mercenaria *from two geographic locations (n = 24 from each group)*

	Buzzard's Bay	Great Bay
Total cell count (per mm³)	$\bar{x}$ = 1954.9	1411.7
	sd = 1062.7	879.9
Packed cell volume	$\bar{x}$ = 0.46%	0.34%
	sd = 0.27%	0.14%

Each tube was immediately capped and the contents mixed by shaking. Packed cell volumes (percentage of hemolymph volume occupied by cells), total cell counts, differential analyses, and measurements of cell sizes were made using the cells from the latter preparation. Packed cell volume was measured by use of the micro-hematocrit method while total cell counts were obtained with a hemacytometer. Ten cells of each type from 24 clams from each location were measured. All cells, including those observed in stained smear preparations, were measured by use of a calibrated eyepiece micrometer.

Fixed and stained smears

The hemolymph placed on glass slides was permitted to settle for 10 minutes at room temperature (21° ± 1° C), after which the adhered cells were fixed by flooding the slides with 3% seawater-glutaraldehyde. Subsequently, the attached cells were rinsed in distilled water for 2 minutes, dehydrated in 95% ethanol for 1 minute, and air dried. The cells were then stained with 4% Giemsa's stain in Sorenson's buffer, pH 6.5, for 20 minutes, rinsed with fresh buffer followed by distilled water, and air dried.

Estimates of the ratio of nuclear area to total cell area in spread, fixed, and stained cells were obtained by the following method. The length of the cell or nucleus was multiplied by its width and this product for the nucleus was divided

TABLE II

Mean sizes of immediately fixed leucocytes from two groups of Mercenaria mercenaria *of 24 animals each (n = 240 for each cell type from each group). The two types of agranular cells are indistinguishable in immediately fixed preparations. All measurements are given in μm.*

	Great Bay		Buzzard's Bay	
	Length	Width	Length	Width
Granular cells	$\bar{x}$ = 12.10	10.36	13.49	11.73
	sd = 2.61	1.33	1.82	1.89
Agranular cells	$\bar{x}$ = 10.35	8.79	12.02	9.87
	sd = 1.33	1.07	1.43	1.27

TABLE III

Differential counts of immediately fixed and stained spread leucocytes of Mercenaria mercenaria *from two geographical areas. The two types of agranular cells are indistinguishable in immediately fixed preparations.*

	Buzzard's Bay	Great Bay
	Immediately fixed leucocytes	
Granulocytes	$\bar{x}$ = 58.40%	65.70%
	sd = 6.31%	7.87%
Agranular cells	$\bar{x}$ = 41.60%	34.30%
	sd = 6.31%	7.87%
	Stained spread leucocytes	
Granulocytes	$\bar{x}$ = 67.73%	67.61%
	sd = 13.64%	14.47%
Fibrocytes	$\bar{x}$ = 24.07%	19.60%
	sd = 14.33%	6.75%
Hyalinocytes	$\bar{x}$ = 8.27%	12.88%
	sd = 2.29%	14.35%

by the product for the entire spread cell. One hundred cells of each type from clams obtained from the two collection areas were sampled. It is recognized that the nuclear to total cell area ratios obtained by this method are only approximations since the spread cells and nuclei are of various shapes, but for the purpose of comparison are assumed to approach ellipsoids in their average dimensions. Based on this assumption, in ascertaining these ratios, that portion of the area of the rectangle tangential to the four sides not included in the ellipse was subtracted. It is noted that the subtracted area is constant irrespective of whether the actual shape of the included ellipsoid is a circle or a regular ellipse.

All data were processed on a CDC 6400 computer, utilizing a packaged statistics program, the Lehigh Amalgamated Package for Statistics (LEAPS). The coefficient of correlation used was Pearson's Product-Moment.

Fresh cells

Fresh leucocytes obtained from the anterior adductor muscle sinus of several clams from both locations were observed in hanging drops or as sealed wet mounts using brightfield, phase, and Nomarski interference optics. All observations were made at room temperature (21° ± 1° C).

Vital staining

One drop of a 0.01% solution of Janus Green B or neutral red in seawater was added to four drops of fresh hemolymph on slides and a No. 1 coverglass, ringed with petroleum jelly, was gently applied. Cells were observed for up to 1 hour following exposure to the dyes and the uptake of stains by various organelles was observed.

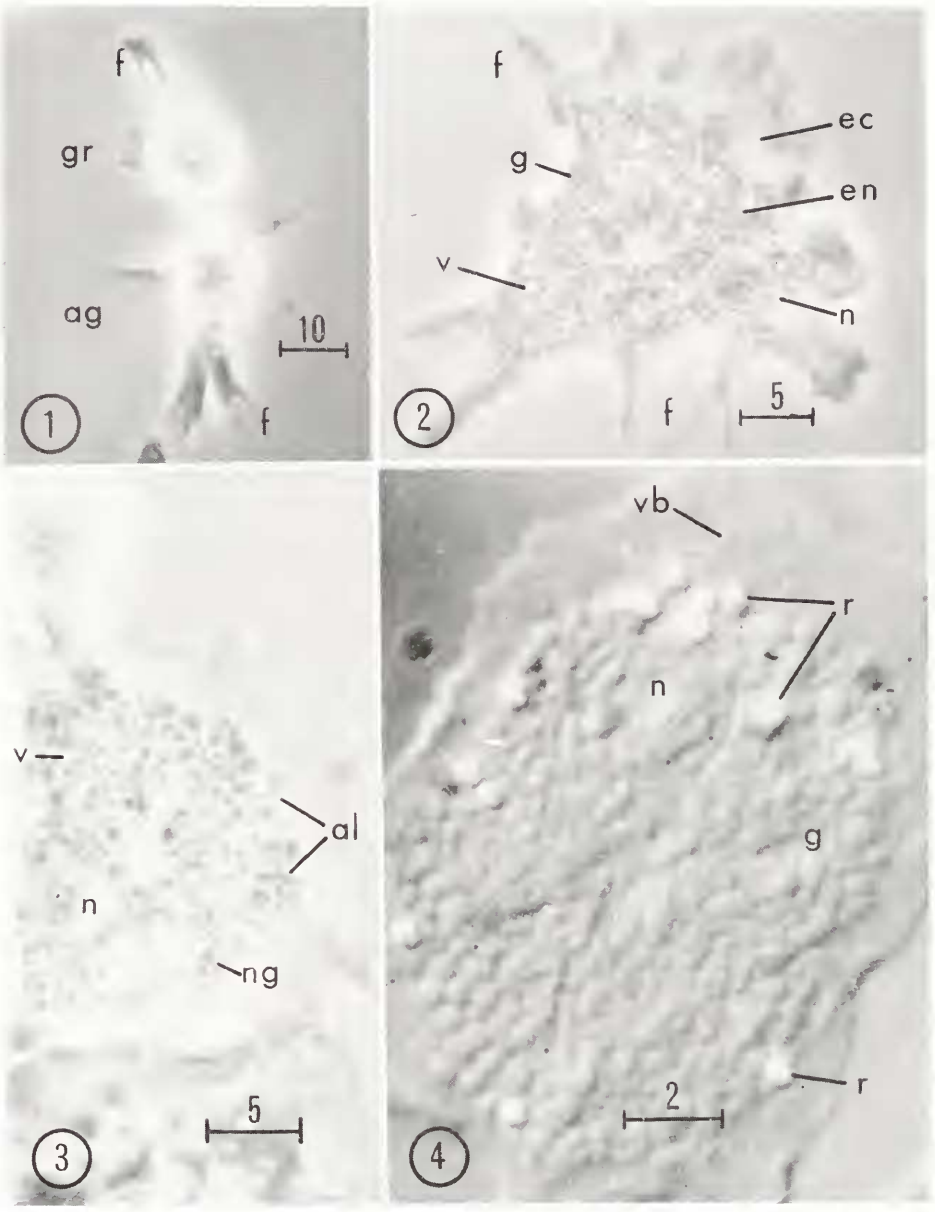

FIGURE 1. Figures 1–4 are living leucocytes of *Mercenaria mercenaria* with scale-bar units in μm; Figure 1, contracted granulocyte (gr) and agranular (ag) leucocytes, with filopodia-like projections (f) (phase contrast).

FIGURE 2. Spread granulocyte showing the separation of endoplasm (en) and ectoplasm (ec), filopodia-like projections (f), and various inclusions including granules (g), vacuoles (v), and nucleus (n) (phase contrast).

FIGURE 3. Spread granulocyte enclosing both normal (ng) and altered (al) granules (phase contrast).

Results

The mean dimensions of the 48 clams from both groups were 8.98 ± 0.55 cm in length, 7.30 ± 0.40 cm in width, and 4.84 ± 0.29 cm in thickness. Based on these measurements, the clams from both groups fall into a small size range.

Total cell counts and packed cell volumes of hemolymph from clams from Buzzard's Bay and Great Bay are included in Table I.

Living leucocytes in vitro

Living leucocytes of *M. mercenaria*, when first placed on slides are mostly irregularly oval or spindle-shaped. Two general varieties of leucocytes, granular and agranular, are easily distinguished by their sizes and appearances in the living, contracted state (Fig. 1). Measurements of contracted leucocytes are given in Table II. Differential analyses of these cells are presented in Table III.

Structurally, both granular and agranular cells include one or more tufts of filopodia-like protrusions (Fig. 1). These tufts are approximately one-half the diameter of the cell in length and move in a three-dimensional sweeping motion.

Nuclei and cytoplasmic inclusions are visible in contracted granular cells but are difficult to define because of the refractiveness of the many cytoplasmic granules present (Fig. 1).

The cytoplasm of granular cells is hyaline and slightly refractile. Few cytoplasmic inclusions are visible except for a few refractile bodies situated near the large, oval nucleus.

Cell adherence and spreading

Upon contacting the slide, both granular and agranular leucocytes adhere and commence to spread. Contact between an attached and a non-attached cell usually results in adhesion of the two. Such cells may be of the same or different types. Occasionally, clumps of from two to several hundred adhered leucocytes can be seen immediately subsequent to slide preparation. Within 5–30 minutes at $21° \pm 1°$ C, many of the cells from these clumps migrate outward over the substrate. It is noted that leucocytes will adhere to and spread on both upper and lower glass surfaces.

Spreading of individual cells begins with adherence to the slide. Cells that do not adhere do not spread.

Spreading of specific cell types

The spreading behavior of *M. mercenaria* leucocytes is of significance in cell identification. Specifically, three types of cells can be distinguished by this feature in conjunction with others. The cells referred to earlier as granular cells are being formally designated as granulocytes while those referred to as agranular cells can be identified as of two types being designated as fibrocytes and hyalinocytes. This

FIGURE 4. Spread granulocyte. Note vermiform body (vb) in the ectoplasm (ec); and the nucleus (n), refractile bodies (r), and granules (g) in the endoplasm (en) (Nomarski interference).

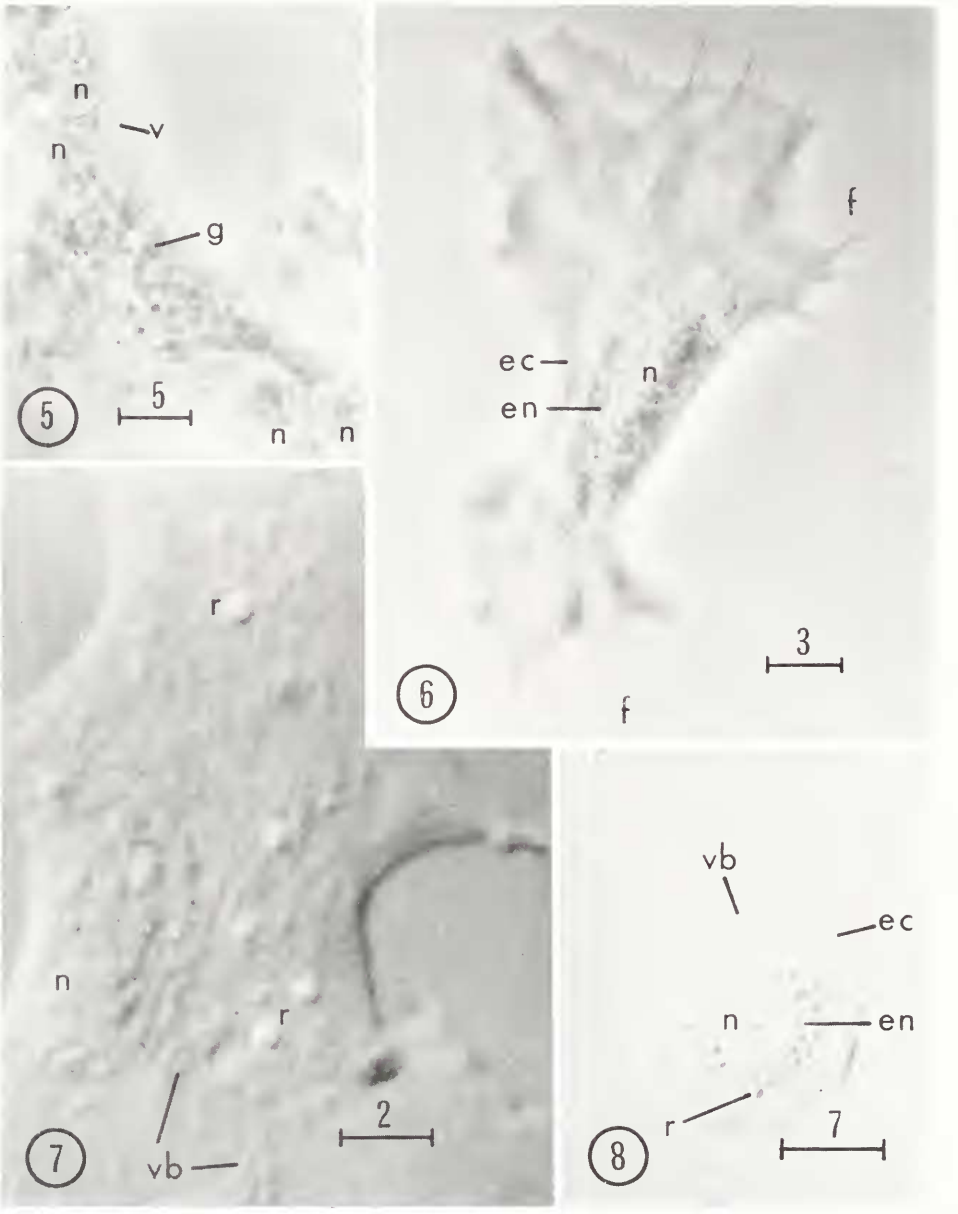

FIGURE 5. Figures 5–8 are living leucocytes of *Mercenaria mercenaria* with scale-bar units in μm; Figure 5, multinucleate, giant granulocyte containing four nuclei (n), granules (g), and vacuoles (v) (phase contrast).

FIGURE 6. Fibrocyte showing ectoplasm (ec) with filopodia-like projections (f), endoplasm (en), and acentric nucleus (n) (phase contrast).

FIGURE 7. Fibrocyte containing refractile bodies (r), nucleus (n), and vermiform bodies (vb) (Nomarski interference).

FIGURE 8. Hyalinocyte showing ectoplasm (ec), endoplasm (en), containing refractile bodies (r), and vermiform bodies (vb) (Nomarski interference).

terminology is being adopted after Foley and Cheng (1972), who have identified morphologically similar cells in the hemolymph of the American oyster, *Crassostrea virginica*. Descriptions of these three types of cells of *M. mercenaria* follow.

Granulocytes. Subsequent to adherence, the cytoplasm of each granulocyte flows out along the substrate in an irregular pattern. At the same time, cytoplasmic refractility decreases and the cytoplasmic inclusions become more visible (Figs. 2, 3). The endoplasm of the spread cell contains at least four types of identifiable inclusions other than the nucleus. These are: (1) vermiform bodies (possibly mitochondria), which are thin and elongate, reaching lengths of about 2 μm (Fig. 4); (2) vacuoles from less than 1 to 4 or 5 μm in diameter and which may contain inclusions (Figs. 2, 3, 5); (3) small, spherical refractile inclusions each averaging 0.7 μm in diameter and which are most readily recognizable when observed with Nomarski interference optics (Fig. 4); and (4) granules, which are the most conspicuous of the inclusions in the granulocytes (Figs. 2–5). Each of these granules averages 0.8 μm in greatest diameter. They are alterable in shape and may vary from being spherical to elongate (Fig. 3).

Surrounding the endoplasm is the hyaline, agranular ectoplasm, the periphery of which is undulating. Beyond this border extend a variable number of spike-like projections, each measuring 1–8 μm in length (Figs. 2, 3).

Spread granulocytes are motile and portray active cyclosis. Occasionally, granulocytes, which appear to have fused with one another, have been observed in preparations of living cells (Fig. 5). The resulting giant, multinucleate cells contain the same cytoplasmic inclusions as those occurring in individual granulocytes. Cyclosis also occurs in these multinucleate cells.

Fibrocytes. Cells of this type are generally spindle-shaped in the contracted state and subsequent to adherence, spread slowly across the substrate with the nucleus usually located along one side (Fig. 6).

These fibrocytes contain motile vermiform bodies (possibly mitochondria), each measuring up to 2 μm in length and which are more or less evenly distributed throughout the cytoplasm (Fig. 7). Small refractile bodies (< 1 μm) generally occur adjacent to the nucleus. In this type of cell the demarcation between the endoplasm and ectoplasm is not as well defined as in granulocytes.

Beyond the periphery of the spread fibrocyte protrude a variable number of filopodial projections, which also can be traced back into the endoplasm (Fig. 6).

Following spreading, small sections of cytoplasm are occasionally detached from the substrate and contract or undulate, thus resulting in the slow movement of the cell across the slide. Cyclosis occurs in spread fibrocytes.

Hyalinocytes. The hyalinocyte spreads slowly outward over the substrate. These cells contain some vermiform bodies (possibly mitochondria), vacuoles, few refractile bodies situated near the large, compact, eccentrically located nucleus, and few or no filopodial projections. Slow cyclosis occurs. The hyalinocytes appear to be the least motile of the three cell types.

Uptake of vital dyes

Granulocytes. The cytoplasmic inclusions designated herein as granules take up neutral red from a concentration of 0.02% within 15 seconds; however, the refractile inclusions do not take up the dye. Neutral red in the concentration employed does not appear to be deleterious to these cells. When exposed to

Janus Green B at a concentration of 0.002%, nearly all of the cytoplasmic granules take up the dye rapidly. Furthermore, after 15 minutes, some granules appear violet or purple, thus indicating possible reduction of this dye to diethyl safranin. The refractile cytoplasmic inclusions also do not take up this dye. When exposed to a mixture of neutral red and Janus Green B at a final concentration of 0.001% for each stain, the cytoplasmic granules appear red. It is noted that there are only a few cytoplasmic inclusions, which are interpreted to be mitochondria, that take on the bluish color imparted by Janus Green B.

Fibrocytes and hyalinocytes. Except in the case of a few isolated cytoplasmic

TABLE IV

Mean dimensions of spread, fixed, and stained leucocytes of Mercenaria mercenaria *($n = 100$ for each cell type from each group). All measurements are given in μm.*

	Mean cell length	Mean cell width	Mean nuclear length	Mean nuclear width
		Great Bay		
Granulocytes	$\bar{x}$ = 35.41	25.71	6.63	4.76
	sd = 7.66	6.38	0.94	0.96
Fibrocytes	$\bar{x}$ = 27.42	20.54	6.41	4.41
	sd = 7.13	5.78	1.54	0.98
Hyalinocytes	$\bar{x}$ = 26.18	21.36	7.87	6.00
	sd = 6.84	5.19	1.51	1.05
		Buzzard's Bay		
Granulocytes	$\bar{x}$ = 32.87	25.96	6.24	4.57
	sd = 7.36	6.75	1.19	0.78
Fibrocytes	$\bar{x}$ = 27.14	20.41	5.82	4.09
	sd = 7.22	5.24	1.32	0.95
Hyalinocytes	$\bar{x}$ = 25.54	21.30	7.60	5.79
	sd = 6.28	5.20	1.47	1.04

inclusions, both of these types of cells do not take up appreciable amounts of either neutral red or Janus Green B, singly or combined, at the concentrations employed.

Staining characteristics

Examination of glutaraldehyde-fixed and Giesma-stained, spread leucocytes verified the presence of the three cell types as based on their nuclear and cytoplasmic morphology and on the ratios of nuclear to total cell areas (Tables IV and V). These distinguishable cell types correspond to the three recognizable in preparations of living cells. The dimensions and differential analyses of the three cell types are presented in Tables III and IV, respectively. The ratios of nuclear to total cell areas are listed in Table V.

Granulocytes. The endoplasm of spread, stained granulocytes contains vacuoles, an eccentrically situated, compact nucelus, and pale blue or pink cytoplasmic granules in a network of intensely stained small (< 1 μm) blue inclusions (Fig. 9).

The pale blue ectoplasm is very finely granular and includes a variable number of spike-like projections, many of which appear to originate in the endoplasm (Fig. 9). Occasionally, granulocytes with multiple nuclei are observed (Fig. 10).

Fibrocytes. The cytoplasm of fibrocytes contains an interlacing, loose array of fibrous material that is most conspicuous in the ectoplasm. Small blue inclusions are situated at the junction of these fibers. The endoplasm is slightly vacuolar and contains the compact, eccentrically situated nucleus. From the periphery of the fan-shaped ectoplasm spread a variable number of spike-like projections, which in some instances originate in the endoplasm (Fig. 11).

Hyalinocytes. The vacuolar, pale-blue cytoplasm is spread subcircularly around the eccentrically located, large oval nucleus. The endoplasm stains more darkly than the ectoplasm and a diffuse gradient occurs between the two zones. Few or no projections occur along the periphery (Fig. 12).

TABLE V

Ratios of mean nuclear area to mean total cell area of fixed and stained spread leucocytes from two groups of Mercenaria mercenaria *($n = 100$ cells of each type from each group); z tests, degrees of freedom (df) = 99, $P < 0.01$; Great Bay: $A = B$, $z = 5.94$; $A = C$, $z = 12.19$; $B = C$, $z = 7.63$. Buzzard's Bay: $A = B$, $z = 3.63$; $A = C$, $z = 12.65$; $B = C$, $z = 7.86$. z test for same type of cells from the two groups: $df = 99$, $P > 0.05$. Granulocytes, $z = 0.60$; fibrocytes, $z = 1.44$; hyalinocytes, $z = 0.57$.*

	Great Bay	Buzzard's Bay
A. Granulocytes	$\bar{x} = 0.0387$	0.0373
	sd = 0.0169	0.0163
B. Fibrocytes	$\bar{x} = 0.0567$	0.0507
	sd = 0.0253	0.0331
C. Hyalinocytes	$\bar{x} = 0.0944$	0.0911
	sd = 0.0426	0.0393

DISCUSSION

Although several earlier studies on the leucocytes of *M. mercenaria* are available (Zacks, 1955; Zacks and Welsh, 1953; Janoff and Hawrylko, 1964), no attempt has been made thus far to classify or distinguish between the morophological types of leucocytes found in the hemolymph of this clam or to determine whether or not hemolymph cells from *M. mercenaria* from different geographic locations are similar. Instead, the earlier investigators have been concerned with the most conspicuous type of cell, the granulocyte, and have largely ignored the other types of cells present in the hemolymph.

The results reported herein indicate the occurrence of three morphological types of leucocytes. In addition, it is now known that these leucocytes are similar, both qualitatively and quantitatively, in clams taken from two geographic locations (Tables IV and V).

Because the clams used in this study were all very similar in size, we feel that it is valid to compare hemolymph cells from both groups of clams.

The high variability in total cell counts undoubtedly reflects differences in the total numbers of circulating leucocytes in individual clams. Previous studies sup-

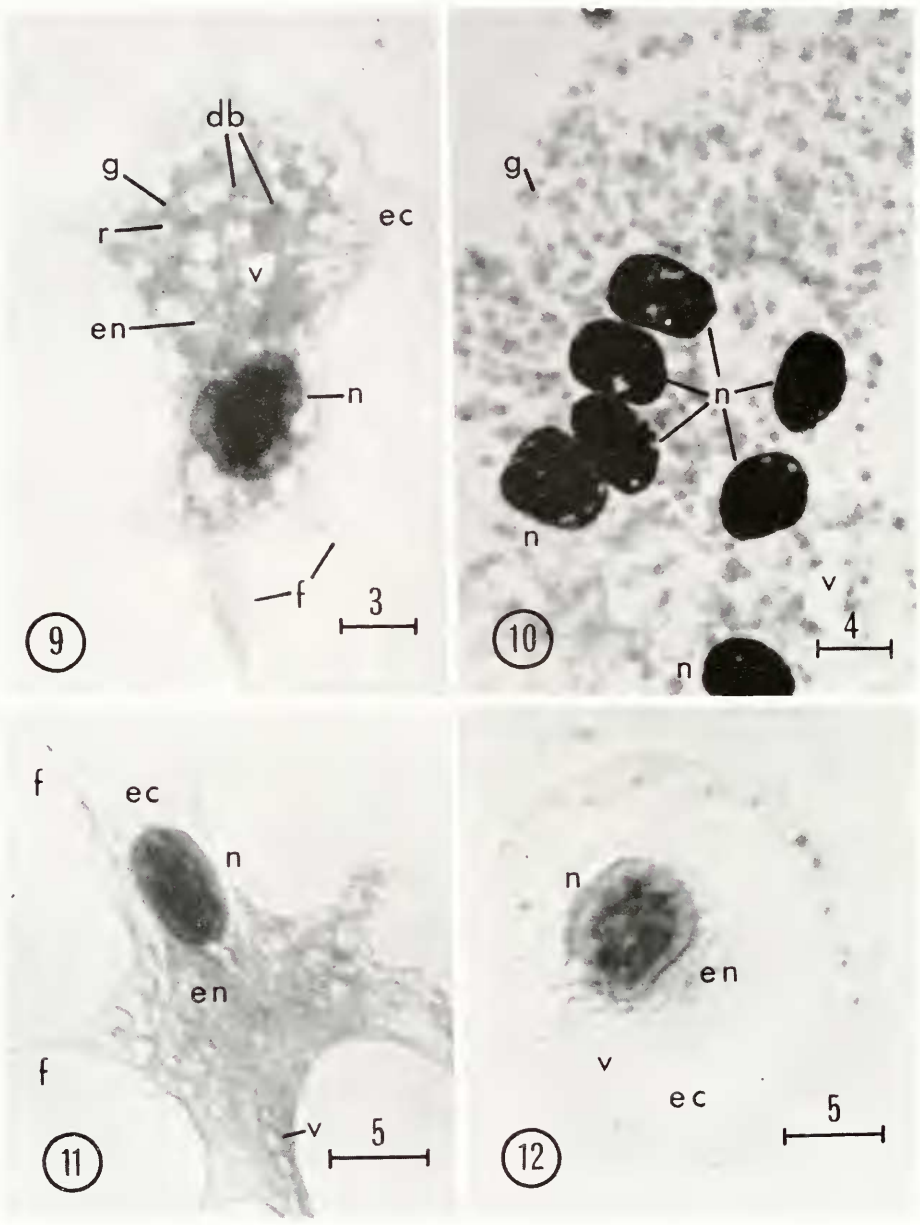

FIGURE 9. Figures 9–12 are fixed and stained leucocytes of *Mercenaria mercenaria*. All cells were fixed with glutaraldehyde except that shown in Figure 10 which was fixed with methanol. Scale-bar units are in μm; Figure 9, granulocyte with filopodia-like projections (f) showing ectoplasm (ec), endoplasm (en) containing refractile bodies (r), vacuoles (v), nucleus (n), and granules (g) with blue inclusions (db) between them (Giemsa stain).

FIGURE 10. Multinucleate granulocyte with seven visible nuclei (n), cytoplasmic granules (g), and vacuoles (v) (Giemsa stain).

port this hypothesis. Specifically, Feng (1965a) has observed that in another estuarine pelecypod, *Crassostrea virginica,* the number of circulating leucocytes is dependent upon the amount of turbulence produced by cardiac action. In addition, he has proposed that numbers of leucocytes may also vary with the feeding and excretion cycles since leucocytes participate in both of these activities. It is thus of interest to note that Feng (1966b) has determined that there is a diurnal cycle of feeding activity in *M. mercenaria* and that while this cycle can be disrupted by changes in intensity of light, temperature, and salinity, or by the presence of organic materials, individual clams may remain inactive, *i.e.*, not pumping, for several days. Thus, variability of circulating leucocytes in the clams may be tentatively attributed to differences in the physiological state of the individuals.

Coefficients of correlation computed between the dimensions of each individual clam and the differential counts, total cell counts, and packed cell volumes are all very small. In addition, coefficients of correlation comparing number of granulocytes with packed cell volumes and total cell counts are also very small. It appears, therefore, that these hemotologic characteristics of leucocytes are independent of the size of the individual clam and are also independent of one another.

As noted, the ratios of nuclear to total cell areas (Table V) are approximate but are included to provide a relative comparison of cell spreading and nuclear size among the three cell types in both groups of clams. With this consideration in mind, it is noted that the means of these ratios for the three cell types within each sample are significantly different using the z test as presented by Tate (1965) ($P < 0.01$), while the means of ratios for cells of the same type are not significantly different using the same test ($P > 0.05$) (Table V). From these observations it is possible to draw certain conclusions. First, the same cell types from clams collected from different areas appear to have the same nuclear size and spreading characteristics, and therefore, we assume that the same leucocytes occur in specimens of *M. mercenaria* from all different geographical populations. In addition, the extreme separation of the means of the ratios obtained from the different cell types suggests that these three cell types are distinct (Table V). However, until further experiments designed to answer questions relative to the origin and ontogeny of molluscan leucocytes are conducted, no definitive statement can be made pertaining to the interrelationships between the granulocytes, hyalinocytes, and fibrocytes of *M. mercenaria.*

Regarding the vital staining of inclusions in leucocytes, Zacks and Welsh (1953) and Zacks (1955) have stated that those inclusions in the granulocytes of *M. mercenaria* that Zacks (1955) has designated as "specific granules," are atypical mitochondria. Zacks (1955) based this assumption on three tests: (1) the uptake of Janus Green B, a traditional mitochondrial vital stain; (2) a positive reaction for phospholipids; and (3) the presence of dehydrogenase activity as determined by the Nitro Blue Tetrazolium test. Zacks also has stated that those vacuoles in

FIGURE 11. Fibrocyte with filopodia-like projections from the ectoplasm (ec), and containing nucleus (n), and vacuoles (v) in the endoplasm (en) (Nomarski interference; Giemsa stain).

FIGURE 12. Hyalinocyte showing ectoplasm (ec) with no filopodia-like projections; and endoplasm (en), with nucleus (n), and a few vacuoles (v) (Nomarski interference; Giemsa stain).

leucocytes that take up neutral red are not the same as the granules that take up Janus Green B. His opinion was based on differences in the appearance of these organelles. Zacks has also reported that the neutral red vacuoles are not preformed in the granulocyte, but form as the dye accumulates in the cells.

To the contrary, on the basis of our observations on the uptake of these two vital dyes by the granulocytes of *M. mercenaria*, we find that the inclusions referred to earlier in this paper as granules, which we believe to be identical to those designated by Zacks (1955) as "specific granules," take up both Janus Green B and neutral red. We have not observed a separate, large population of granules selectively stained with Janus Green B when a mixture of the two stains was used. Thus, we are presently of the opinion that there is only one type of cytoplasmic granule in *M. mercenaria* and it is stained with both Janus Green B and neutral red.

Relative to the selective settling of molluscan leucocytes, Prowse and Tait (1969) have reported that only certain leucocytes of *Helix aspersa* adhere to glass. However, we have found as a result of differential counting of *M. mercenaria* leucocytes in fresh and fixed hemolymph preparations on slides where the cells had been permitted to adhere prior to fixation and staining, that the results are approximately the same (Table III). It thus must be concluded that cells of all three types are capable of adhering to glass.

Leucocytes not only adhere to glass but also to one another. Although there appears to be no true coagulation in the hemolymph of *M. mercenaria*, agglutination of the leucocytes occurs *in vitro* and is more pronounced if the leucocytes are mixed with seawater or with homologous serum. Agglutination observable to the naked eye commonly occurs in these cases. Microscopical observations have shown that leucocytes that make contact *in vitro* will generally adhere to one another.

Also associated with the phenomenon of cell agglutination is the occasional fusion of granulocytes to form multinucleate cells. That true fusion does occur is supported by the observation that during cyclosis the granules and nuclei from all of the participating cells move in the large cytoplasmic mass. The occurrence of multinucleate cells has been reported in *Crassostrea gigas* by Sparks and Pauley (1964) and in *Helisoma duryi normale* by Cheng and Galloway (1970). In both of these instances however, the molluscs had been subjected to injury.

Relative to the adherence and spreading of leucocytes, it is possible that the spike-like processes formed with fans of ectoplasm stretched between them may serve a protective function. These processes generally occur on granulocytes and only rarely on hyalinocytes. Earlier, Bang (1961) had reported that the filopodial processes of leucocytes of oysters are responsible for the capture of microorganisms. Such microorganisms, according to Bang, also appear to be trapped by sticking in the nets of ectoplasm. A similar function may occur in the case of clam leucocytes.

With the establishment of three distinct types of cells in the hemolymph of *M. mercenaria* and having quantified some of the hematologic parameters, it is now possible to attempt to answer some important basic questions relative to the role of these cells in internal defense against foreign materials.

We would like to thank Frank W. Koko, Jr., co-author of the LEAPS program, for his valuable assistance with the statistical analyses.

Summary

The hematological parameters of *Mercenaria mercenaria* of similar size from two geographical areas as well as the morphology and behavior of their leucocytes were studied. On the basis of qualitative and quantitative characteristics, three types of leucocytes, designated as granulocytes, fibrocytes, and hyalinocytes, can be distinguished in both living and stained preparations.

A correlation matrix computed between all parameters considered has revealed an insignificant correlation between the dimensions of whole animal and the differential count, packed cell volume, and total cell count as well as an insignificant correlation between the hematological parameters themselves. The only exception is that there is a positive correlation between the packed cell volume and the total cell count.

LITERATURE CITED

Arcadi, J. A., 1968. Tissue response to the injection of charcoal into the pulmonate gastropod *Lehmania poirieri*. *J. Invert. Pathol.*, **11**: 59–62.

Bang, F. B., 1961. Reaction to injury in the oyster (*Crassostrea virginica*). *Biol. Bull.*, **121**: 57–68.

Cheng, T. C., 1967. Marine Molluscs as Hosts for Symbioses: With a Review of Known Parasites of Commercially Important Species. Volume 5 in F. S. Russell, Ed., *Advances in Marine Biology*. Academic Press, New York.

Cheng, T. C., and P. C. Galloway, 1970. Transplantation immunity in molluscs: The Histoincompatibility of *Helisoma duryi normale* with allografts and xenografts. *J. Invert. Pathol.*, **15**: 177–192.

Cheng, T. C., and E. Rifkin, 1970. Cellular reactions in marine molluscs in response to helminth parasitism. Pages 443–496 in S. F. Snieszko, Ed., *Symposium on Diseases of Fishes and Shellfishes*. American Fisheries Society Special Publication No. 5, Washington, D. C.

Cheng, T. C., A. S. Thakur and E. Rifkin, 1969. Phagocytosis as an internal defense mechanism in the Mollusca: With an experimental study of the role of leucocytes in the removal of ink particles in *Littorina scabra* Linn. Pages 546–563 in *Proceedings of the Symposium on Mollusca*, Part II. Marine Biological Association, India.

Feng, S. Y., 1959. Defense mechanisms of the oyster. *Bull. N. J. Acad. Sci.*, **4**: 17.

Feng, S. Y., 1965a. Heart rate and leucocyte circulation in *Crassostrea virginica* (Gmelin). *Biol. Bull.*, **128**: 198–210.

Feng, S. Y., 1965b. Pinocytosis of proteins by oyster leucocytes. *Biol. Bull.*, **129**: 95–105.

Feng, S. Y., 1966a. Experimental bacterial infections in the oyster *Crassostrea virginica*. *J. Invert. Pathol.*, **8**: 505–511.

Feng, S. Y., 1966b. Biological aspects of hard clam purification. *Proc. Nat. Shellfish. Ass.*, **56**: 3.

Feng, S. Y., 1967. Responses of molluscs to foreign bodies with special reference to the oyster. *Fed. Proc.*, **26**: 1685–1692.

Feng, S. Y., J. S. Feng, C. N. Burke and L. H. Khairallah, 1971. Light and electron microscopy of the leucocytes of *Crassostrea virginica* (Mollusca: Pelecypoda). *Z. Zellforsch. Mikrosk. Anat.*, **120**: 222–245.

Foley, D. A., and T. C. Cheng, 1972. Interaction of molluscs and foreign substances: The morphology and behavior of hemolymph cells of the American oyster, *Crassostrea virginica*, in vitro. *J. Invert. Pathol.*, **19**: 383–394.

Janoff, A., and E. Hawrylko, 1964. Lysosomal enzymes in invertebrate leucocytes. *J. Cell. Comp. Physiol.*, **63**: 267–271.

Pauley, G. B., and S. M. Krassner, 1972. Cellular defense reactions to particulate materials in the California sea hare, *Aplysia californica*. *J. Invert. Pathol.*, **19**: 8–17.

Prowse, R. H., and N. N. Tait, 1969. In vitro phagocytosis by amoebocytes from the haemolymph of *Helix aspersa* (Muller). I. Evidence for opsonic factor(s) in serum. *Immunology*, **17**: 437–443.

Sparks, A. K., and G. Pauley, 1964. Studies of the normal postmortem changes in the oyster, *Crassostrea gigas* (Thunberg). *J. Invert. Pathol.*, **6**: 260–268.

Stauber, L. A., 1950. The fate of India ink injected intracardially into the oyster, *Ostrea virginica* Gmelin. *Biol. Bull.*, **98**: 227–241.

Tate, M. W., 1965. *Statistics in Education and Psychology*. Collier-Macmillan Limited, London.

Tripp, M. R., 1958a. Disposal by the oyster of intracardially injected red blood cells of vertebrates. *Proc. Nat. Shellfish. Ass.*, **48**: 143–147.

Tripp, M. R., 1958b. Studies on the defense mechanism of the oyster. *J. Parasitol.*, **44** (sect. 2): 35–36.

Tripp, M. R., 1960. Mechanisms of removal of injected microorganisms from the American oyster, *Crassostrea virginica* (Gmelin). *Biol. Bull.*, **119**: 210–223.

Tripp, M. R., 1961. The fate of foreign materials experimentally introduced into the snail *Australorbis glabratus*. *J. Parasitol.*, **47**: 745–751.

Zacks, S. I., 1955. The cytochemistry of the amoebocytes and intestinal epithelium of *Venus mercenaria* (Lamellibranchiata), with remarks on a pigment resembling ceroid. *Quart. J. Microscop. Sci.*, **96**: 57–71.

Zacks, S. I., and J. H. Welsh, 1953. Cholinesterase and lipase in the amoebocytes, intestinal epithelium and heart muscle of the quahog, *Venus mercenaria*. *Biol. Bull.*, **105**: 200–211.